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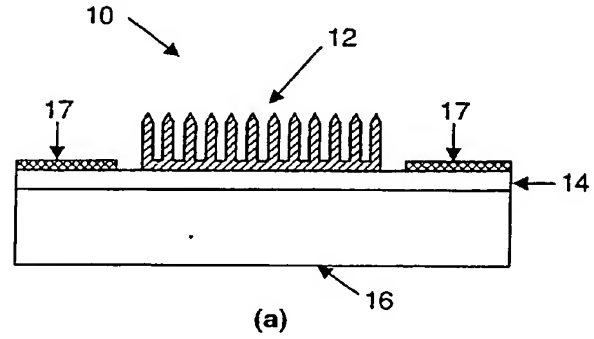
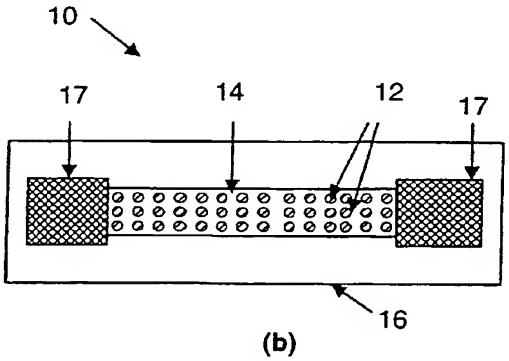
(54) Title: MULTIFUNCTIONAL BIOSENSOR BASED ON ZnO NANOSTRUCTURES





(57) Abstract: The present invention provides the multifunctional biological and biochemical sensor technology based on ZnO nanostructures. The ZnO nanotips (12) serve as strong DNA or protein molecule binding sites to enhance the immobilization. Patterned ZnO nanotips are used to provide conductivity-based biosensors (10). Patterned ZnO nanotips are also used as the gate (26) for field-effect transistor (FET) type sensors (20). Patterned ZnO nanotips are integrated with SAW (30) or BAW (56) based biosensors. These ZnO nanotip based devices operate in multimodal operation combining electrical, acoustic and optical sensing mechanisms. The multifunctional biosensors can be arrayed and combined into one biochip, which will enhance the sensitivity and accuracy of biological and biochemical detection due to strong immobilization and multimodal operation capability. Such biological and biochemical sensor technology are useful in detection of RNA-DNA, DNA-DNA, protein-protein, protein-DNA and protein-small molecules interaction. It can be further applied for drug discovery, and for environmental monitoring and protection.

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MULTIFUNCTIONAL BIOSENSOR BASED ON ZnO NANOSTRUCTURES

[0001] This invention was made with Government support under Grant Nos. NSF ECS-0088549 and NSF CCR-0103096, awarded by the National Science Foundation. Therefore, the Government has certain rights in this invention.

CROSS-REFERENCE TO RELATED APPLICATION:

[0002] This application claims priority to United States Provisional Patent Application No. 60/385,884, which was filed on June 6, 2002.

FIELD OF THE INVENTION

[0003] This invention relates generally to biosensor technology, and pertains more particularly to novel multifunctional biosensors based on zinc oxide (ZnO) nanostructures for biological, biochemical, chemical and environmental applications.

BACKGROUND OF THE INVENTION

[0004] The nanoscale science and engineering have shown great promise for the fabrication of novel nano-biosensors with faster response and higher sensitivity than that of planar sensor configurations, due to their small dimensions combined with dramatically increased contact surface and strong binding with biological and chemical reagents which could have important applications in biological and biochemical research, as well as in environmental monitoring and protection.

[0008] Particularly, it is an objective of this invention to provide conductivity-based biosensors using semiconductive or conductive ZnO nanotips; to provide field-effect-transistor (FET)-based biosensors by using ZnO nanotips as the gate of the FET; to provide surface acoustic wave (SAW)-based biosensors by integrating ZnO nanotips into SAW devices to form highly sensitive and multichannel biosensors; and to provide bulk acoustic wave (BAW)-based biosensors by integrating ZnO nanotips into BAW devices to form highly sensitive and multichannel biosensors.

[0009] As ZnO nanotips can be made semiconducting, transparent and conducting, or piezoelectric, their unique electrical, optical and acoustic properties can serve as the basis for multifunctional sensors. A sensor chip comprising of arrays and combinations of various types of ZnO nanotip-based biosensors also allow for multimodal operation.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] Fig. 1a shows a schematic of a vertical cross-section view of the device structure for the conductivity-based ZnO nanotip biosensor.

[0011] Fig. 1b shows a schematic of top view of the conductivity-based ZnO nanotip biosensor structure.

[0012] Fig. 2 shows a schematic of a vertical cross-section of a ZnO nanotip gate metal-insulator-semiconductor field effect transistor (MISFET).

[0013] Fig. 3a shows a schematic of a vertical cross-section view of a ZnO nanotip SAW sensor.

the biological element. Biological recognition *in vivo* at a single cell level is characterized by high sensitivity, fast response, specificity and reversibility.

[0021] A "sensor surface" refers to the location upon which a binding partner is immobilized for the purpose of measuring changes in physical properties, such as optical refractive index, electrical conductivity, mass loading, etc. They include, but are not limited to, semiconductor, metal and dielectric surfaces.

[0022] ZnO is a wide bandgap semiconductor having a direct bandgap of 3.32eV at room temperature and can be made semiconducting, piezoelectric, ferroelectric, ferromagnetic, and transparent and conducting through proper doping. ZnO has an exciton binding energy of 60 meV. It is found to be significantly more radiation hard than silicon (Si), gallium arsenide (GaAs), and GaN.

[0023] ZnO is a polar semiconductor with the (0002) planes being Zn-terminated and the $(000\bar{2})$ planes being O-terminated. These two crystallographic planes have opposite polarity and hence have different surface relaxations energies. This leads to a higher growth rate along the c-axis. The ZnO film grown on many semiconducting, insulating or metallic substrates have a preferred c-axis orientation normal to the surface. Therefore, ZnO growth results in a pillar like structure called ZnO nanotips on these semiconducting, insulating and metallic substrates, while ZnO grown on R-plane sapphire substrates results in a smooth epitaxial film. The ZnO nanotips can be grown at relatively low temperatures, giving ZnO a unique advantage over other wide bandgap semiconductor nanostructures, such as GaN and SiC.

($LiNbO_3$), in which case the conductivity-based sensor can be integrated with the SAW-based sensor to realize another type of multifunctional sensor as described later in this application.

[0028] The conductive thin film 14 has certain conductivity, and it can be a semiconductor, such as Si with properly designed doping level, a metallic thin film, such as gold (Au), a transparent conductive oxide, such as indium tin oxide (ITO), or even the multilayer thin film. The thin film and the metal bond pads are deposited on the substrate 16, then patterned using the standard microelectronic processing techniques.

[0029] The ZnO nanotips 12 can be deposited on the substrate 16 and thin film 14 using the technology, but not limited to metal-organic chemical vapor deposition (MOCVD), then patterned by the standard photolithography and etching process.

[0030] These ZnO nanotips serve as DNA or protein molecule binding sites. In other words, the ZnO nanotips 12 are preferably bonded with protein or DNA molecules to make conductivity-based biosensors, as will be described in detail below. Specifically, the conductive thin film 14 surface, with ZnO nanotips 12 grown on the top, will be designed and fabricated as conductivity-based biosensors. Preferably a probe is attached to said tip to seek the targeted molecule due to bioreaction. The probe may preferably be attached on a binding site or a target molecule preferably has a probe. Any useful probes preferably such as chemiluscence, fluorescence, etc. The dimensions of the conductive pattern, the aspect ratio and doping level of the ZnO nanotips, are optimized to enhance the sensitivity. Due to depletion or accumulation of carriers in the nanotips as a result of bioreactions, the conductance of the patterned tip arrays will change significantly. The depletion (accumulation) of the nanotips will result in a transient current across the line. The amplitude

which can be used with biosensors. The first is a metal-insulator-semiconductor FET (MISFET), composed of a metal gate deposited on a gate insulator layer, which is deposited on the semiconductor. The second is a metal-semiconductor FET (MESFET), composed of a metal gate directly deposited on the semiconductor. If the gate insulator is specifically an oxide, the MISFET device is known as a metal-oxide-semiconductor FET (MOSFET).

[0033] An FET type of biosensor can be realized by depositing ZnO nanotips on the gate region of the FET. Such an FET can be a current existing Si MOSFET, GaAS MESFET, etc. The surface charge changes occurring with the target on the ZnO nanotips will result in a potential difference between the gate and the substrate, and modulate the current flowing between the source and the drain. Unlike the resistor-type conductivity-based sensor described above, the FET type sensor can be used for both transient and steady-state current measurements, making it a more flexible device.

[0034] More specifically, a novel transparent FET sensor is composed of a ZnO nanotip gate and a ZnO FET. Referring to Figure 2, there is shown a schematic of a vertical cross-section view of nanotip gate ZnO MISFET biosensor 20. It is composed of a R-plane sapphire (R-Al_2O_3) substrate 22, a semiconductor ZnO thin epitaxial layer as a channel 24, doped ZnO source and drain regions 25, a gate insulator 26, metal electrodes 27 to the source and drain regions 25, the ZnO nanotips 12 deposited on the gate, and an encapsulation layer 28 to protect the device except the nanotip gate area.

[0035] In this device, n^+-ZnO 25 regions serve as the source and the drain. When Al is used for the metal contacts 27, it will heavily dope the ZnO thin film 24 under it, resulting in good non-alloyed ohmic contact as developed in H. Sheng, N.W. Emanetoglu, S.

of a top view respectively of ZnO nanotip SAW biosensor 30. The ZnO nanotip SAW biosensor is composed of a piezoelectric substrate 32, an insulating amorphous layer 34, a metal input interdigital transducer (IDT) 36, a metal output IDT 38, and the ZnO nanotips 12.

[0039] The piezoelectric substrate 32 can be, but is not limited to, quartz, $LiNbO_3$, lithium tantalate ($LiTaO_3$), etc. An insulating amorphous layer 34 is deposited on the piezoelectric substrate and patterned using the standard microelectronic processing techniques. This insulating amorphous layer can be, but is not limited to, SiO_2 or Si_3N_4.

[0040] The ZnO nanotips 12 are deposited on the surface of the insulating layer 34 using MOCVD, or other deposition technology, then patterned and etched to define the nanotip coverage area. The metal IDTs 36 and 38 are then deposited and patterned using standard microelectronic processing techniques. The metal of choice is Al, but other metals can also be used.

[0041] The ZnO nanotip SAW sensor device 30 operates similarly to a planar SAW biosensor. A dualchannel biosensor consisting of two identical devices, one without target coating serving as the reference and the other with target coating serving as the sensor, are used together. As the target binds with the ZnO nanotips 12 on the sensor device, mass loading of the sensor will result in a decrease of the phase velocity under the ZnO nanotips. This will results in a phase difference between the output signals of the reference and the sensor devices. The use of ZnO nanotips dramatically enhances the immobilization of DNA, protein and other small biomolecules, therefore the sensitivity of the biosensors. Our preliminary experimental results demonstrate that the immobilization rate of ZnO nanotips is over thirty times higher than that of smooth surface. It is well known that the rough surface

and 3μm apart from each electrode for both IDTs. The phase velocity (v) of the SAW on the 128° Y-cut $LiNbO_3$ is 3668m/s, and the wavelength (□) of the test pattern is 12μm. From the equation $f_c = v/$□ the expected center frequency is 305MHz. The bandwidth is BW_{3db} = $(0.9/N_p)^* f_c = 0.9*305/50 = 5.49$MHz. On the propagation path of the prototype devices, the sensor region has 600nm ZnO nanotip/100nm SiO_2 is 1116 μm long and 594 μm wide. Furthermore, the dual channel (reference and sensor channels) device is tested using an Agilent 8573D Network Analyzer. The reference channel has no protein bonding and the sensor channel is bonded with 100 ng protein on the ZnO nanotip over an area of $6.629\times10^{-3}cm^2$.

[0046] The frequency responses of the reference sensor device and the actual test sensor device are shown in a graph shown in Fig. 4a. The X-axis is the frequency and the Y-axis is the S_{21} transmission spectra of reference and sensor device. As shown in Fig. 4a, the sensor device has a shift to lower frequency compared with the reference device. An additional insertion loss of 6.14 dB is observed for the protein bonded sample. However, the insertion loss shift depends on a number of factors, and by itself is not a good sensing mechanism. Instead, the phase shift of the signal is preferred for accurate and repeatable measurements.

[0047] Fig. 4b shows a graph displaying a phase difference between the reference and sensor prototype devices. The X-axis is the frequency and Y-axis is the phase difference between the reference and sensor device. As shown in Fig. 4b, the sensor device has a 47.68° phase shift at the center frequency 305MHz compared with the reference device. The phase shift increases with increasing frequency, due to the different velocity dispersion characteristics of the SAW propagating in the reference and sensor channels. The phase

[0051] In a further embodiment of the ZnO nanotip BAW sensor 50, the center area of the top surface of the piezoelectric substrate is not metallized. The ZnO nanotips 12 are deposited on the bare piezoelectric substrate surface 52, so that the top metal electrode 54 surrounds, but does not contact the ZnO nanotips 12.

[0052] The ZnO nanotip BAW sensor 50 operates similarly to a BAW resonator device. The BAW resonator will resonate at a specific frequency determined by the piezoelectric substrate material properties and thickness. When bonding of the target occurs on the ZnO nanotips 12, mass-loading results with a shift in the resonance frequency of the resonator, directly proportional to the amount of target material bonded to the ZnO nanotips 12.

[0053] In a further embodiment of the ZnO nanotip BAW sensor 50, the crystal resonator of Fig. 5 can be replaced with a thin film resonator structure, including, but not limited to, air gap resonators, solidly mounted resonators and membrane (Film Bulk Acoustic Resonator or FBAR) resonators. The thin film resonator structure includes, but not limited to, an air-gap structure on top surface of the substrate, a membrane structure on the substrate and an acoustic mirror on top surface of the substrate.

[0054] In a further embodiment of the present invention, there is disclosed a biochip consisting of ZnO nanotip array as biosensors to simultaneously detect a number of different biological information. For certain clinical and scientific applications it is desirable to use multiple biosensors on a chip for simultaneous detection of several biomolecular targets. For this purpose a biosensor chip having multiple detection units for different targets. As

of avidin as disclosed by S. Tombelli, M. Mascini, L. Braccini, M. Anichini, and A.P. Turner, *"Coupling of a DNA piezoelectric biosensor and polymerase chain reaction to detect apolipoprotein E polymorphisms", Biosens Bioelectron.* **15**, 363 (2000). Thiolated DNA oligonucleotides are covalently attached to mercaptosilane-derivatised surface via succinimidyl 4-[malemido-phenyl]butyrate (SMPB) crosslinker as disclosed by T.A. Taton, C.A. Mirkin, and R.L. Letsinge, *"Scanometric DNA array detection with nanoparticle probes", Science* **289**, 1757 (2000) and L.A. Chrisey, G.U. Lee, and C.E. O'Ferrall, *"Covalent attachment of synthetic DNA to self-assembled monolayer films", Nucleic Acids Res.* **24**, 3031 (1996). The Thiolated DNA oligonucleotides can serve as the biological recognition elements which recognize the analytes.

[0058] For testing the efficiency of DNA immobilization procedures, model oligonucleotides with a radioisotope/fluorescent label are preferably used. For testing and calibration of DNA/RNA ZnO nanotip biosensors, a series of complementary pairs of oligonucleotides (20 and 50 nucleotides in length) which are 30, 50 and 70% GC-reach are synthesized having different percents of complementarity (from no to several mismatches). Basically, preferably light with a particular wavelength (λ) is passed through the transparent ZnO nanotip 12 and one member of each pair of oligonucleotides is immobilized on a surface of ZnO biosensor 10 and the device is tested in a series of hybridization experiments with the corresponding targets. Different hybridization conditions, as well as different RNA targets are evaluated. These targets may preferably be labeled. As an example of the practical application two sets of experiments are conducted with specific targets. One such target is detection of cold-shock inducible cspA mRNA from *E.coli.* Some major advances in understanding of the regulatory mechanisms of cspA expression have been made as disclosed by S. Phadtare, J. Alsina, and M. Inouye, *"Cold-shock response and cold-shock proteins",*

immobilization method for SAW-biosensors: covalent attachment of antibodies via CNBr", Biosens Bioelectron **14**, 93 (1999).

[0060] In another case, for protein immobilization, a histidine kinase called EnvZ was used. The protein was first phosphorylated with γ-[^{32}P] ATP and solution of ^{32}P-labled EnvZ was loaded on the nanotips grown on a square glass plate (5x5 mm). After incubation for 90 minutes, plates were washed extensively at room temperature by changing the washing buffer solution five times. The radioactivity of the plate was then measured. More than 90% of the protein was retained on the surface, indicating a strong affinity of the protein to the ZnO surface. It was also found that EnvZ attached on the nanotip surface retained its biochemical property, as approximately 80% of ^{32}P radioactivity was released when the plate was incubated in a solution containing OmpR, an EnvZ substrate, but no radioactivity was released in the presence of other proteins. This model system is preferably used for analysis of protein-protein interactions via optical techniques, since EnvZ forms a stoichiometric complex with OmpR.

[0061] Fluorescence resonance energy transfer (FRET) is a quantum mechanical process wherein excitation energy is transferred from a donor fluorophore to an appropriately positioned acceptor positioned acceptor fluorophore without emission of a photon. Energy can be transferred this way only over a very limited distance, and the efficiency of the energy transfer varies inversely with the sixth power of the distance separating the donor and acceptor. One of the most important uses of FRET spectroscopy is to study protein-protein interactions.

[0063] The ZnO DNA and protein nanotip array based sensors in this application will further benefit to explore genome-wide gene expression for molecular diagnostics, drug target discovery, and validation of drug effects. The ZnO nanotip-based biosensors can also be applied to development of new methods for the prevention, diagnosis and treatment of diseases. Furthermore, the application of ZnO and its nanostructure-based biosensors can be extended to detection of toxic biochemical agents and hazardous chemicals against bioterrorism and environmental monitoring and protection. Unlike other sensor technologies, ZnO biosensors can operate in multimodes due to its multifunctional material properties (semiconductor, piezoelectric, transparent and conductive, etc.). Nanotips made from ZnO and its ternary compound can be used for UV absorption and fluorescence detection. ZnO nanotip arrays can be highly dense for diagnostic kits and flow-through systems, including ZnO UV biotesting bench (containing emitters, detectors, modulators, and filters), gene chip, lab-on-a chip and living-cell chip.

[0064] While the invention has been described in relation to the preferred embodiments with several examples, it will be understood by those skilled in the art that various changes may be made without deviating from the fundamental nature and scope of the invention as defined in the appended claims.

7. The biosensor device of claim 1 wherein said substrate is transparent, thereby allowing said device to be operable in optical mode simultaneously with the conductivity mode.

8. The biosensor device of claim 7 further comprising:

magnesium zinc oxide nanotips deposited on said transparent substrate for UV absorption biosensing, wherein upon illumination of UV light on said device, change in UV absorption is detected due to a biological or biochemical reaction of the immobilized DNA or protein molecules or small biomolecules on the ZnO nanotips with corresponding targeted DNA or protein molecules.

9. The biosensor device of claim 7 further comprising:

a layer of gold coated on the ZnO nanotips for fluorescence biosensing wherein upon illumination of light on said device, the fluorescence is detected due to a biological or biochemical reaction of the immobilized DNA or protein molecules or small biomolecules on the ZnO nanotips with corresponding targeted DNA or protein molecules or small biomolecules.

10. The biosensor device of claim 1 operates in multiple modes due to multifunctional material properties of the ZnO nanotips such as semiconducting, piezoelectric, or transparent and combinations thereof.

11. A ZnO nanotip-gate field effect transistor biosensor device comprising:

a semiconductor FET structure including a source region, a drain region and a gate region;

ZnO nanotips deposited on top of said gate dielectric layer to serve as the ZnO nanotip-gate; and

an encapsulating layer deposited on surface of the device where there are no ZnO nanotips.

17. The transparent FET biosensor device of claim 16 wherein said substrate is insulating substrate and transparent to the wavelength less than 240nm.

18. The device of claim 16 wherein said semiconductor epitaxial layer is a wide-band gap semiconductor material comprising AlN, GaN, $Al_xGa_{1-x}N$, SiC, ZnO, $Mg_xZn_{1-x}O$, or their heterostructures and combinations thereof.

19. The device of claim 16 wherein said ZnO nanotips serve as DNA, protein molecule or small biomolecule binding sites to detect presence of the DNA, protein molecules or small biomolecules to be targeted.

20. The device of claim 19wherein upon said detection of the targeted molecules, surface charge of the ZnO nanotip-gate changes, causing a change in conductance of said channel, thereby resulting in a change in current between the source and the drain regions.

21. The device of claim 16 wherein said substrate, wide band gap semiconductor, and ZnO nanotip-gate are transparent, thereby allowing said device to be operable in optical mode simultaneously with electrical mode.

22. The device of claim 16 wherein said semiconductor epitaxial layer is ZnO.

a substrate;

a semiconductor epitaxial layer grown on top of said substrate;

a source region, a drain region and a channel said regions situated in the semiconductor epitaxial layer;

a metal gate layer deposited on top of said channel region, resulting in a Schottky contact to said channel region;

metal electrodes deposited on top of said source and drain regions;

ZnO nanotips deposited on top of said metal gate layer to serve as the ZnO nanotip-gate; and

an encapsulating layer deposited on surface of said device where there are no ZnO nanotips.

31. The device of claim 30 wherein said semiconductor epitaxial layer is a wide-band gap semiconductor material comprising AlN, GaN, $Al_xGa_{1-x}N$, SiC, ZnO, $Mg_xZn_{1-x}O$, or their heterostructures and combinations thereof.

32. The device of claim 30 wherein said ZnO nanotips serve as DNA, protein molecule or small biomolecule binding sites to detect presence of the DNA, protein molecules or small biomolecules to be targeted.

33. The device of claim 30 wherein upon said detection of the targeted molecules, surface charge of the ZnO nanotip-gate changes causing a change in the conductance of said channel, thereby resulting in a change in current between the source and the drain regions.

34. The device of claim 30 wherein the said semiconductor epitaxial layer is ZnO.

ZnO nanotips serve as DNA, protein molecule or small biomolecule binding sites, and metal input and output interdigital transducers deposited on the substrate.

42. The device of claim 41 wherein upon binding of the targeted molecules with said ZnO nanotips causes a mass loading on the SAW path resulting in a decrease of phase velocity under said ZnO nanotips.

43. The device of claim 41 wherein said ZnO nanotips enhance binding strength and immobilization of the targeted DNA, protein molecules or small biomolecules.

44. The device of claim 41 wherein said piezoelectric substrate is transparent and the device is operable in optical mode simultaneously with the SAW mode.

45. The device of claim 41 wherein upon illumination of UV light on said device, the change in UV absorption is detectable due to a biological and biochemical reaction of sensor layer of immobilized DNA, protein molecules on the ZnO nanotips with targeted DNA, protein molecules or small biomolecules.

46. The device of claim 41 wherein said insulating layer is replaced with a resistor-type conductive layer and the device is operable in electrical mode simultaneously with SAW mode.

47. The device of claim 46 wherein said piezoelectric substrate is replaced with a layered structure composed of a piezoelectric layer on a non-piezoelectric substrate.

54. The device of claim 52 wherein said ZnO nanotips enhance binding strength and immobilization of the DNA, protein molecules or small biomolecules.

55. The device of claim 52 wherein said piezoelectric layer comprises a single crystal piezoelectric substrate.

56. The device of claim 52 wherein said piezoelectric layer comprises a piezoelectric thin film.

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57. A ZnO nanotip biochip comprising arrays of ZnO nanotip conductivity mode biosensor devices, ZnO nanotip-gate FET biosensor devices and ZnO nanotip SAW or BAW biosensor devices, and combinations thereof.

58. The biochip of claim 56 wherein said sensors can be operated simultaneously in conductivity mode, optical mode, SAW mode or BAW mode, and combinations thereof.

59. A method for detecting biological molecules comprising:

providing a conductivity mode ZnO nanotip biosensor including ZnO nanotips having binding sites including at least one biological probe;

exposing said binding site to a sample having a potential target molecule;

detecting a change in conductivity in said ZnO nanotips, wherein said change in conductivity being indicative of a chemical and biochemical reaction of the potential target molecule and the biological probe.

63. A method for detecting biological molecules comprising:

providing a ZnO based BAW sensor including ZnO nanotips having binding sites including at least one biological probe;

exposing said binding site to a sample having a potential target molecule;

detecting a change in resonance frequency of the sensor, wherein said change in resonance frequency being indicative of a chemical and biochemical reaction of the potential target molecule and the biological probe.

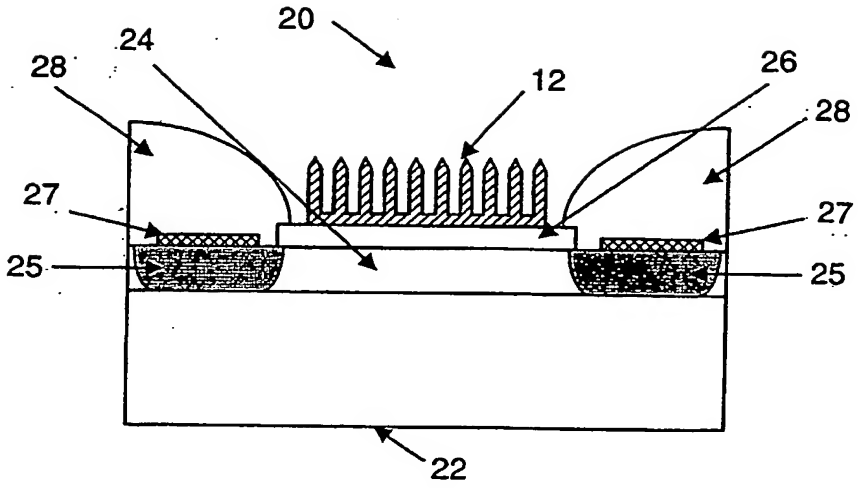
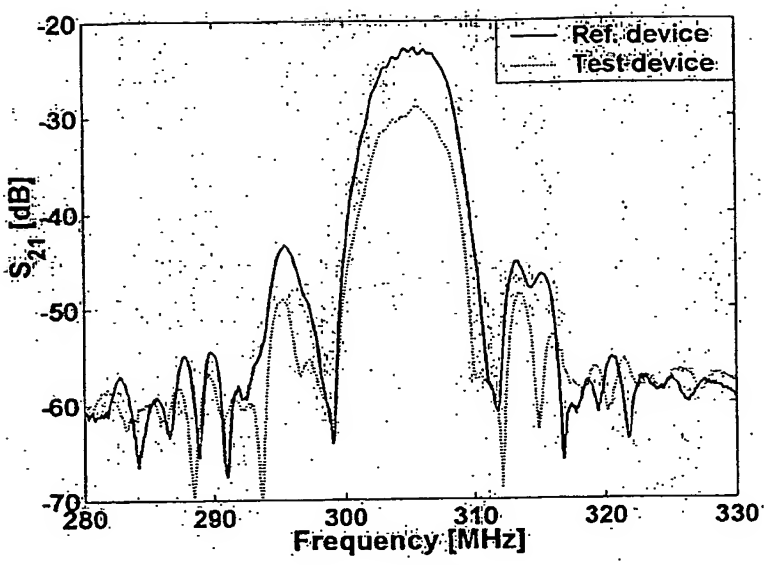


Fig. 2


Ref. device
Test device
S_{21} [dB]
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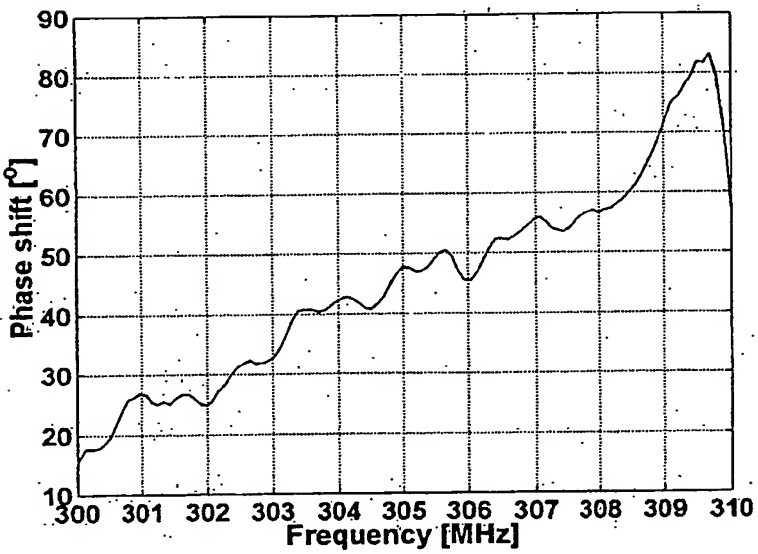
-30
-40
-50
-60
-70
280
290
300
310
320
330
Frequency [MHz]

(a)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30
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10
300
301
302
303
304
305
306
307
308
309
310
Frequency [MHz]

(b)

Fig. 4

INTERNATIONAL SEARCH REPORT

PCT/US 03/17822

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N27/327 G01N27/12 G01N27/00 G01N29/02 C12Q1/68
G01N27/414

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, EPO-Internal, PAJ, INSPEC, COMPENDEX, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WO 99 35312 A (VAYSSIERES L ET AL) 15 July 1999 (1999-07-15) page 12, line 20 -page 13, line 23 page 21, line 18 -page 23, line 26 figures 3,4A,4B; example 1 ---	1,2,4-7, 10,59 8,9, 11-40,60
Y	EMANETOGLU N W ET AL: "MgxZn1-xO: a new piezoelectric material" 2001 IEEE ULTRASONICS SYMPOSIUM PROCEEDINGS, vol. 2, 7 October 2001 (2001-10-07), pages 253-256, XP010584520 ISBN 0-7803-7177-1	8
A	the whole document ---	18,24, 31,36

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 September 2003	22/10/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Johnson, K

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	YUAN W ET AL: "Current - voltage properties of piezoelectric thin film ZnO in a micromechanical force sensor" 1988 IEEE ULTRASONICS SYMPOSIUM PROCEEDINGS, vol. 1, 5 October 1998 (1998-10-05), pages 593-596, XP010330956 ISBN 0-7803-4095-7 the whole document	52-56,63
P,A	MUTHUKUMAR S ET AL: "Selective MOCVD growth of ZnO nanotips" IEEE TRANSACTIONS ON NANOTECHNOLOGY, vol. 2, no. 1, March 2003 (2003-03), pages 50-54, XP002256223 ISSN 1536-125X cited in the application	

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

1. The multiple definitions of the invention given in the independent claims 1, 11, 16, 30, 41, 52, 57 (apparatus) and claims 59-63 (methods) are such that the claims as a whole are inconcise. In any case, the claims lack clarity as a whole, because the only partially overlapping subject matter of the claims makes it difficult to establish the essential features of the invention. This is particularly true of claims 11, 16 and 30, which all appear to relate to the same embodiment based on a chemically sensitive field effect transistor, or CHEMFET. This places an undue burden on others seeking to establish the extent of protection afforded by the claims. Hence the subject matter of the claims is not clearly defined, in breach of Article 6 PCT.

2. The problem is compounded because a biological probe bound to the ZnO nanotips would appear to be an essential feature of the independent method claims but an equivalent feature has been omitted from the independent apparatus claims. Thus a contradiction arises which engenders further doubt regarding the scope of protection afforded by the claims and adds to the lack of clarity.

3. Furthermore, in dependent claims 5, 6, 8, 9, 10-13, 19, 20, 27, 28, 32, 33, 39, 40, 42, 45, 53, 58, directed to preferred embodiments of the apparatus, the subject matter is defined more by how the apparatus is operated rather than in terms of the structural features needed to define the apparatus clearly. So these claims also contravene Article 6 PCT. Claims 43 and 54 seek protection for an effect, namely that '... ZnO nanotips enhance binding strength and immobilization of the targeted DNA, protein molecules or small biomolecules', for which the applicants have produced not the slightest support in description and drawings of the application as filed. Consequently, they lack support in further contravention of Article 6 PCT.

4. Therefore the claims are so unclear and so lacking in support that a meaningful search over the whole scope claimed was impossible. Instead, the search was limited to the following subject matter:

(i) A ZnO nanotip biosensor device comprising:
- a biological recognition element; and
- a signal transducer,

CHARACTERISED in that

- the biological recognition element is constituted by ZnO nanotips deposited on the sensing surface of the transducer, said ZnO nanotips having binding sites including at least one biological probe.

(ii) A method of detecting biomolecules using such a ZnO nanotip biosensor device comprising the steps of:
- exposing said binding site to a sample having a potential target molecule; and
- detecting a change in the signal of the biosensor indicative of a chemical or biochemical reaction of the potential traget molecule and the

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